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ORIGINAL RESEARCH

The Association Between Azathioprine Genetic Polymorphisms, Clinical Efficacy and Adverse Drug Reactions Among Egyptian Patients with Autoimmune Diseases

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Purpose: The study aimed to detect the frequencies of allelic variants (*TPMT*3A*, *TPMT*3C*, and *TPMT*3G*) in the *TPMT* genes in the Egyptian population and assess the association between *TPMT* polymorphisms and azathioprine (AZA)—clinical efficacy and adverse drug reactions among Egyptian patients with autoimmune diseases.

Design: A prospective, observational single-center clinical trial.

Setting: Rheumatology and Rehabilitation Department, Kasr Alainy University Hospital, Faculty of Medicine, Cairo University.

Patients: Patients attending Kasr Alainy Rheumatology Outpatient Clinic between December 1, 2017 and June 30, 2019 were included in the study after signing a consent form. *TPMT* genetic polymorphisms were detected for all patients, and the association between polymorphisms presence and azathioprine's clinical efficacy and adverse drug reactions were determined.

Results: A total of 150 patients with a mean age of 35.85 years were enrolled in this study. About 72% of patients were heterozygous in the *TPMT*3* G460A and *TPMT*3* A719G mutant alleles and 81% were wild type in the *TPMT*2* G238C mutant allele. Abnormal liver function tests were detected in 42% of patients. Myelosuppression was presented as anemia which was detected in 63% of patients, leucopenia in 51%, and thrombocytopenia in 25% of patients. AZA clinical failure has occurred in 50% of patients where AZA was discontinued or shifted to another drug which occurred in 45% of patients. Myelosuppression rates were higher in homozygous patients in the three mutant alleles, but statistically significant in *TPMT*2* G238C while not statistically significant in *TPMT*3* G460A and *TPMT*3* A719G. Females had a higher risk of immunosuppression than males (p-value 0.031).

Conclusion: The study provided an overview of the genomic variations in the Egyptian population. Routine TPMT genotyping prior to the initiation of AZA therapy should be considered.

Keywords: azathioprine, genetic polymorphisms, autoimmune diseases, Egyptian patients

Introduction

Autoimmune disorders are a group of heterogeneous diseases that occur when the body tissues are attacked by its own immune system.[1] During the pathogenesis of these disorders, both T cells and B cells are activated even without any precipitating cause or infection.[2] The guidelines for treating these disorders aimed to change the thresholds of immune activation by using immunosuppressants.[3] Azathioprine

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(AZA) is used in the treatment of various autoimmune diseases including systemic lupus erythematosus (SLE). Despite AZA efficacy in treating manifestations of autoimmune diseases, its use is associated with myelosuppression, gastric toxicity, pancreatitis, and liver toxicity. Interindividual variability to AZA effect and outcomes is related to its genetic polymorphism. AZA is activated by the liver to 6-mercaptopurine (6-MP) through a glutathione-dependent process.[4]

Thiopurine S-methyltransferase (*TPMT*) is the enzyme responsible for catalyzing the methylation of 6-MP. It competes with xanthine oxidase and hypoxanthine-guanine phosphor-ribosyl-transferase to detect the percent of 6-MP metabolized to the active metabolite 6-thioguanine nucleotide (6-TGN) metabolite: the main component responsible for immunosuppressive effect.[5] Many studies have proved that there are many genetic polymorphisms in the gene encoding for *TPMT*. Recently, the number of mutations in this specific gene reached 37 mutations.[6] Patients were categorized into: (i) intermediate *TPMT* activity which is heterozygous for a mutant *TPMT* allele represents 4–11% of patients. (ii) very low or absent *TPMT* activity which is homozygous or compounds heterozygous about 1 in 300 patients.[7–9] Intermediate *TPMT* activity patients can accumulate 50% more from 6-TGNs in comparison with patients that have normal or high *TPMT* activity. These individuals will have a high risk of adverse drug reactions (ADRs) caused by AZA. High doses of 6-TGNs will be accumulated in patients with deficient *TPMT* activity which may result in fatal myelosuppression.[10] Another study concluded that very few patients who have two inactive *TPMT* alleles are homozygous deficient, experienced severe bone marrow suppression; a large percentage of those who are heterozygous encounter moderate to severe bone marrow suppression and those who are homozygous for wild type *TPMT* alleles have reduced the risk of bone marrow suppression.[11] Detecting *TPMT* genotypes or phenotypes before starting AZA therapy is documented in many updated treatment protocols.[11–13] In autoimmune diseases, patients with homozygous wild type should receive full initial doses, while heterozygous patients required lower doses (30–70% of the recommended dose) and substantially very small doses or other drugs are required for the rare homozygous-deficient patients.[14] TPMT protective testing has a great advantage as the doses can be tolerated on the basis of *TPMT* status. This decreases the probability of acute myelosuppression without affecting disease progression.[15] To our knowledge, only one study investigated the frequency of *TPMT* gene polymorphisms (*TPMT*3A* and *TPMT*3C* alleles) in Egypt.[16] However, no studies investigated the frequency of *TPMT*3G* or the association between *TPMT* polymorphisms and AZA clinical efficacy and ADRs among Egyptian patients with autoimmune diseases. This study aimed to detect the frequencies of allelic variants (*TPMT*3A*, *TPMT*3C* and *TPMT*3G*) in the *TPMT* genes among Egyptian population and to assess the association between *TPMT* polymorphisms and AZA clinical efficacy and ADRs in Egyptian patients with autoimmune diseases.

Materials and Methods

Design

A prospective, observational single-center clinical trial.

Setting

Rheumatology and Rehabilitation Department, Kasr Alainy University hospital, Faculty of Medicine, Cairo University.

Patients

Patients attending Kasr Alainy Rheumatology Outpatient Clinic between December 1, 2017 and June 30, 2019 was included in the study after signing a consent form. Patients were diagnosed as having an autoimmune/chronic inflammatory disease; systemic lupus erythematosus, Behçet's disease, mixed connective tissue disease, rheumatoid arthritis, and scleroderma during the study period were included in the study after signing a consent form. *TPMT* genetic polymorphisms were detected for all patients and the association between the presence of polymorphisms, clinical efficacy of AZA and adverse drug reactions were determined.

Exclusion Criteria

Patients were excluded from the study if they had myelosuppression or abnormal liver function tests before receiving AZA.

DNA Isolation and Analysis of *TPMT* Gene Polymorphisms

A volume of 5 mL of EDTA anticoagulated whole blood was obtained from each patient, genomic DNA was then extracted from peripheral blood nucleated cells. *TPMT* gene polymorphisms were detected by using real time-

polymerase chain reaction (RT-PCR). All samples were analyzed to detect the G238C (rs 1800462) transversion in *TPMT*2*, the G460A (rs 1800460), and A719G (rs 1142345) transitions in *TPMT*3* alleles. Genotypes were determined by using a TaqMan® genotyping assay.

Clinical Data Collection and Patient Follow-up

All patients' medical files were reviewed to detect the AZA doses and its associated ADRs as well as any change in the treatment plans. Follow-up of all patients continued until the end of the study (April 30, 2020).

Myelosuppression Laboratory Values Used During Analysis

Neutropenia: ANC (absolute neutrophil count) <2000 cells/mm^3

Mild neutropenia: ANC between 1000 and 1500 cells/mm^3

Moderate neutropenia: ANC between 500and 1000 cells/mm^3

Severe neutropenia: ANC <500 cells/mm^3

Thrombocytopenia: a platelet count <100000/µL

Mild anemia: hemoglobin between 9.5 and 10.9 g/dL

Moderate anemia: hemoglobin between 8 and 9.4 g/dL

Severe anemia: hemoglobin between 6.5 and 7.9 g/dL

Life-threatening anemia: hemoglobin <6.5 g/dL

Statistical Analysis

Data were analyzed using IBM SPSS Statistics Version 22. Data were tested for normality using the Kolmogorov–Smirnov test and the Shapiro–Wilk test. Quantitative data were presented as mean and standard deviation while qualitative data were presented as number and percentage. Analysis of variance test was used to compare numerical variables between the different categories of SNPs. Chi-squared test or Fisher's exact test as appropriate were used to compare categorical variables between the different categories of SNPs. *P*-value set significant at 0.05 levels. All tests were two-tailed.

Ethical Approval

The study was approved by the ethical committee for grants of the British University in Egypt.

This study was conducted in accordance with the Declaration of Helsinki.

Results

This study investigated genetic polymorphism in the *TPMT* gene in a total of 150 patients with a mean age of 35.85 years. Table 1 shows the demographics and patients' characteristics. The study determined the prevalence of three mutant alleles; *TPMT*3* G460A (rs 1800460), *TPMT*2* G238C (rs 1800462), and *TPMT*3* A719G (rs 1142345). About 72% of patients were heterozygous in the *TPMT*3* G460A and *TPMT*3* A719G mutant alleles and 81% were wild type in the *TPMT*2* G238C mutant allele as shown in Table 1. Seventy percent of patients' diagnoses were systemic lupus erythematosus. A dose of 100 mg (1*2=one 50 mg tablet *2) was the initial dose in 94% of patients and about 75% of patients were maintained on the same dose. Abnormal liver function tests were detected in 42% of patients. Myelosuppression was represented as anemia which was detected in 63% of patients, leucopenia in 51%, and thrombocytopenia in 25% of patients. The AZA-clinical failure occurred in 50% of patients where AZA discontinuation or shift to another drug (in 45% of patients).

Table 2 shows the frequencies of homozygous, heterozygous, and wild type carriers of a mutant allele of the *TPMT*3* G460A. Myelosuppression was presented as anemia and leucopenia and there was a statistically significant difference between the homozygous, heterozygous and wild type carriers of the mutant allele of the *TPMT*3* G460A as shown in Table 2. There was also a statistically significant difference between the three types in regard to the initial and maintenance doses, the severity of anemia as well as the treatment shift parameter.

Table 3 shows the frequencies of homozygous, heterozygous, and wild type carriers of the mutant allele of the *TPMT*2* G238C. Myelosuppression was presented as anemia and leucopenia and there was a statistically significant difference between the homozygous, heterozygous, and wild-type carriers of the mutant allele of the *TPMT*2* G238C as shown in Table 3. There was also a statistically significant difference between the three types in regard to the severity of anemia as well as the treatment shift parameter.

Table 4 shows the frequencies of homozygous, heterozygous, and wildtype carriers of the mutant allele of the *TPMT*3* A719G. Abnormal liver function tests (LFTs) were presented as elevation in the aspartate transaminase (AST) and alanine transaminase (ALT) and there was a statistically significant difference between the

Table 1 Demographics and Patients Characteristics

		N=150	%
Age/year (mean ±SD)		**35.85±10.21**	
Sex	Male	36	24.0
	Female	114	76.0
*TPMT*3* G460A (rs1800460)	Homozygous	34	22.7
	Heterozygous	107	71.3
	Wild type	9	6.0
*TPMT*2* G238C (rs1800462)	Homozygous	19	12.7
	Heterozygous	9	6.0
	Wild type	122	81.3
*TPMT*3* A719G (rs1142345)	Homozygous	27	18.0
	Heterozygous	107	71.3
	Wild type	16	10.7
Diagnosis	SLE	105	70.0
	MCTD	7	4.7
	RA	5	3.3
	Behçhet's	20	13.3
	Scleroderma	4	2.7
	Others	9	6.0
Initial dose	1*1	9	6.0
	1*2	141	94.0
Maintenance dose	1*2	112	74.7
	1*3	38	25.3
Abnormal LFTs	No	88	58.7
	Yes	62	41.3
Anemia	No	56	37.3
	Yes	94	62.7
Leucopenia	No	73	48.7
	Yes	77	51.3
Thrombocytopenia	No	112	74.7
	Yes	38	25.3
D/C and resume[a]	No	75	50.0
	Yes	75	50.0
Treatment shift[b]	No	83	55.3
	Yes	67	44.7

Notes: 1*1=one tablet 50 mg once daily, 1*2=one tablet 50 mg twice daily, 1*3=one tablet 50 mg three times daily. [a]D/C and resume: discontinue AZA and resume again during the treatment course. [b]Treatment shift: shift AZA to another drug. Others= dermatomyositis: two patients, polymyositis: two patients, Sjogren's syndrome: two patients, Wegner's granulomatosis, Cogan's syndrome, and Takayasu arteritis: one patient for each disease.

Abbreviations: LFTs, liver function tests; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis.

homozygous, heterozygous and wild type carriers of the mutant allele of the *TPMT*3* A719G as shown in Table 4. There was also a statistically significant difference between the three types in regard to the treatment shift parameter. Myelosuppression rates were higher in homozygous patients in the three mutant alleles, but it was statistically significant in *TPMT*2* G238C while not statistically significant in *TPMT*3* G460A and *TPMT*3* A719G. Females had a higher risk of immunosuppression than males (p-value 0.031) as shown in Table 5.

Discussion

This current study detected the frequencies of three mutant *TPMT* alleles; *TPMT*3* G460A, *TPMT*2* G238C, and *TPMT*3* A719G. About 72% of patients were heterozygous in the *TPMT*3* G460A and *TPMT*3* A719G mutant alleles and 81% were wildtype in the *TPMT*2* G238C mutant allele. These findings are consistent with the results of many other studies conducted by Kubota and Chiba; Kumagai et al; and Hiratsuka et al, these studies showed that in Caucasians *TPMT*3A* (460G>A: rs1800460 and 719A>G: rs1142345) represents the most frequent allele, whereas *TPMT*3C* (719A>G: rs1142345) represents the most frequent allele in the Japanese population.[17–19] In this current study, *TPMT*3* G460A, *TPMT*2* G238C, and *TPMT*3* A719G are the most common defective alleles of the *TPMT* gene examined. About 23% of patients were homozygous in the *TPMT*3* G460A mutant allele; the rates of heterozygous and wild type were 71% and 6%, respectively. The percentages of homozygous, heterozygous and wild type in the mutant allele *TPMT*2* G238C was 13, 6, and 81%, respectively, while these rates were 18, 71, and 11% in the *TPMT*3* A719G mutant allele.

Our results were discordant with a study conducted on 194 Egyptian patients done by Hamdy et al that concluded that the frequency of wild type allele was 97% and 3% of patients who were heterozygous for one of the mutant alleles. *TPMT*1/TPMT*3A* was detected in one patient, while *TPMT*1/TPMT*3C* was detected in five subjects. Neither G238C (*TPMT*2*), G460A alone (*TPMT*3B*) nor the homozygous type of any mutant allele was determined in the Egyptian population. Their study also concluded that *TPMT*3C* is the inherited *TPMT* variant allele in the Egyptian population, accounting for 86% of the variant alleles detected. *TPMT*3A* is the second most recurrent variant and accounts for the remaining 14% of the *TPMT* variant alleles in Egyptians.[16]

In comparison with other global populations across Asia, Europe and the Americas, the gene variant that occurs most frequently in Caucasians is *TPMT*3A* (approximately 85%), *2, *3B and *3C occur much less frequently. In Africans and African Americans, as in Caucasians, *1 is the wild type, but

Table 2 AZA Clinical Efficacy and Associated ADRs in Different Types of *TPMT *3* G460A Mutant Allele

		***TPMT*3* G460A (rs1800460)**							
		Homozygous		**Heterozygous**		**Wild Type**		**Test**	***P*-value**
		N=34	**22.66%**	**N=107**	**71.33%**	**N=9**	**6.00%**		
Sex	Male Female	4 30	11.8 88.2	31 76	29.0 71.0	1 8	11.1 88.9	5.060**	0.091
Diagnosis	SLE MCTD RA Behçet's Scleroderma Others	26 2 0 2 2 2	76.5 5.9 0.0 5.9 5.9 5.9	70 5 5 18 2 7	65.4 4.7 4.7 16.8 1.9 6.5	9 0 0 0 0 0	100 0.0 0.0 0.0 0.0 0.0	7.867**	0.545
Initial dose	1*1 1*2	4a, b 30a, b	11.8 88.2	3b 104b	2.8 97.2	2a 7a	22.2 77.8	8.141**	0.020***
Maintenance dose	1*2 1*3	31a 3a	91.2 8.8	76a, b 31a, b	71.0 29.0	5b 4b	55.6 44.4	7.386**	0.025***
Abnormal LFTs	No Yes	17 17	50.0 50.0	67 40	62.6 37.4	4 5	44.4 55.6	2.492**	0.321
Anemia	No Yes	8a 26a	23.5 76.5***	42 a, b 65 a, b	39.3 60.7***	6b 3b	66.7 33.3***	6.248**	0.04***
Degree of anemia	Mild Moderate Severe Life-threatening	2a 12a 9a 3a	7.7 46.2 34.6 11.5	30b 19a 14a 2a	46.2 29.2 21.5 3.1	1a, b 1a 1a 0a	33.3 33.3 33.3 0	15.530**	0.005***
Leucopenia	No Yes	8 26	23.5 76.5	60 47	56.1 43.9	5 4	55.6 44.4	11.348**	0.003***
Thrombocytopenia	No Yes	25 9	73.5 26.5	81 26	75.7 24.3	6 3	66.7 33.3	0.388**	0.850
D/C and resume[a]	No Yes	18 16	52.9 47.1	53 54	49.5 50.5	4 5	44.4 55.6	0.295**	0.921
Treatment shift[b]	No Yes	8a 26a	23.5 76.5	69b 38b	64.5 35.5***	6 a, b 3 a, b	66.7 33.3***	18.043**	<0.001***

Notes: 1*1=one tablet 50 mg once daily, 1*2=one tablet 50 mg twice daily, 1*3=one tablet 50 mg three times daily. a, b: Variables sharing same letters are not statistically differ from each other. [a]D/C and resume: discontinue AZA and resume again during the treatment course. [b]Treatment shift: shift AZA to another drug. Others= dermatomyositis: two patients, polymyositis: two patients, Sjogren's syndrome: two patients, Wegner's granulomatosis, Cogan's syndrome, and Takayasu arteritis one patient for each disease. **Analysis of variance test, Chi-squared test or Fisher's exact test as appropriate, variables sharing different letters are statistically different from each other. P-value set significant at 0.05 levels. ***Difference is statistically significant.

Abbreviations: LFTs, liver function tests; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis.

the most common allele is *TPMT*3C*.[20,21] Regarding the Asian population, *TPMT*3C* is also the most common mutation reported while, *TPMT*3A* does not occur.[22–24] *TPMT* activity, in addition to nudix hydrolase 15 genetics, is a critical factor contributing to thiopurine-induced hematotoxicity.[25] *TPMT* activity differs among patients as about 87% of patients have high *TPMT* activity, 11.1% have intermediate activity, and 0.3% have low or absent activity.[26–28] Patients with normal *TPMT* activity are categorized as the wild type *TPMT*1*.[29] Patients (~1 in 178 to 1 in 3736) who are homozygous-deficient universally develop severe bone marrow suppression; an increased percentage of those who are heterozygous develop moderate-to-severe bone marrow suppression, and those who are homozygous for wild type *TPMT* alleles are at reduced risk of myelosuppression.[11] These results were consistent with our findings as 72.5% of

Table 3 AZA Clinical Efficacy and Associated ADRs in Different Types of *TPMT* 2* G238C Mutant Allele

		TPMT** 2 G238C (rs1800462)							
		Homozygous		**Heterozygous**		**Wild Type**		**Test**	***P*-value**
		N=19	**12.66%**	**N=9**	**6.00%**	**N=122**	**81.33%**		
Sex	Male	3	15.8	1	2.8	32	88.9	1.414**	0.482
	Female	16	84.2	8	7.0	90	78.9		
Diagnosis	SLE	14	73.7	7	77.8	84	68.9	2.746**	0.991
	MCTD	1	5.3	0	0.0	6	4.9		
	RA	1	5.3	0	0.0	4	3.3		
	Behçet's	2	10.5	1	11.1	17	13.9		
	Scleroderma	0	0.0	0	0.0	4	3.3		
	Others	1	5.3	1	11.1	7	5.7		
Initial dose	1*1	2	10.5	0	11.1	6	4.9	2.320**	0.252
	1*2	17	89.5	7	88.9	116	95.1		
Maintenance dose	1*2	13	68.4	7	77.8	92	75.4	0.600**	0.869
	1*3	6	31.6	2	22.2	30	24.6		
Abnormal LFTs	No	12	63.2	5	55.6	71	58.2	0.205**	0.948
	Yes	7	36.8	4	44.4	51	41.8		
Anemia	No	4a	21.1	7b	77.8	45a	36.9	8.456**	0.011***
	Yes	15a	78.9***	2b	22.2***	77a	63.1***		
Degree of anemia	Mild	1a	6.7	0a, b	0	32b	41.6	13.897**	0.010***
	Moderate	8a	53.3	1a	50.0	23a	29.9		
	Severe	6a	40.0	0a	0	18a	23.4		
	Life-threatening	0a	0	1b	50.0	4a	5.2		
Leucopenia	No	3a	15.8	4a, b	44.4	66b	54.1	10.056**	0.006***
	Yes	16a	84.2***	5a, b	55.6***	56b	45.9***		
Thrombocytopenia	No	16	84.2	7	77.8	89	73.0	0.992**	0.663
	Yes	3	15.8	2	22.2	33	27.0		
D/C and resume[a]	No	8	42.1	4	44.4	63	51.6	0.764**	0.732
	Yes	11	57.9	5	55.6	59	48.4		
Treatment shift[b]	No	7	36.8	4	44.4	72	59.0	5.852**	0.052***
	Yes	12	63.2***	5	55.6***	50	41.0***		

Notes: 1*1=one tablet 50 mg once daily, 1*2=one tablet 50 mg twice daily, 1*3=one tablet 50 mg three times daily. a, b: Variables sharing same letters are not statistically differ from each other. [a]D/C and resume: discontinue AZA and resume again during the treatment course. [b]Treatment shift: shift AZA to another drug. Others= dermatomyositis: two patients, polymyositis: two patients, Sjogren's syndrome: two patients, Wegner's granulomatosis, Cogan's syndrome, and Takayasu arteritis one patient for each disease. **Analysis of variance test, Chi-squared test or Fisher's exact test as appropriate, variables sharing different letters are statistically different from each other. P-value set significant at 0.05 levels. ***Difference is statistically significant.

Abbreviations: LFTs, liver function tests; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis.

homozygous-type carriers of the mutant allele *TPMT*3* G460A developed anemia and leucopenia. Anemia and leucopenia represented 80% and 84% of homozygous-type carriers of the mutant allele *TPMT*2* G238C and 59% for each of them of homozygous-type carriers of the mutant allele *TPMT*3* A719G.

Myelosuppression was presented as anemia, leucopenia, or thrombocytopenia; these rates were higher in homozygous patients in the three mutant alleles, but it was statistically significant in *TPMT*2* G238C while it was not statistically significant in *TPMT*3* G460A and *TPMT*3* A719G. This study showed ADR as well as

Table 4 AZA Clinical Efficacy and Associated ADRs in Different Types of *TPMT* 3* A719G Mutant Allele

		***TPMT*3* A719G (rs1142345)**							
		Homozygous		**Heterozygous**		**Wild type**		**Test**	***P*-value**
		N=27	**18.00%**	**N=107**	**71.33%**	**N=16**	**10.66%**		
Sex	Male Female	4 23	14.8 85.2	30 77	28.0 72.0	2 14	12.5 87.5	3.365**	0.191
Diagnosis	SLE MCTD RA Behçet's Scleroderma Others	20 1 0 3 3 0	74.1 3.7 0.0 11.1 11.1 0.0	73 6 5 16 0 7	68.2 5.6 4.7 15.0 0.0 6.5	12 0 0 1 1 2	75.0 0.0 0.0 6.3 6.3 12.5	14.385**	0.075
Initial dose	1*1 1*2	2 25	7.4 92.6	6 101	5.6 94.4	1 15	6.3 93.7	0.546**	0.864
Maintenance dose	1*2 1*3	24 3	88.9 11.1	77 30	72.0 28.0	11 5	68.8 31.3	3.597**	0.165
Abnormal LFTs	No Yes	9 18	33.3 66.7***	70 37	65.4 34.6***	9 7	65.3 43.8***	9.197**	0.009***
Anemia	No Yes	11 16	40.7 59.3	37 70	34.6 65.4	8 8	50.0 50.0	1.578**	0.486
Degree of anemia	Mild Moderate Severe Life-threatening	3 5 7 1	18.8 31.3 43.8 6.3	29 23 15 3	41.4 23.9 21.4 4.3	1 4 2 1	12.5 50 25 12.5	7.931**	0.194
Leucopenia	No Yes	11 16	40.7 59.3	57 50	53.3 46.7	5 11	31.3 68.8	3.530**	0.171
Thrombocytopenia	No Yes	21 6	77.8 22.2	79 28	73.8 26.2	12 4	75.0 25.0	0.179**	0.954
D/C and resume[#]	No Yes	14 13	51.9 48.1	51 56	47.7 52.3	10 6	62.5 37.5	1.271**	0.531
Treatment shift[##]	No Yes	9a 18a	33.3 66.7***	63b 44b	58.9 41.1***	11a 5a	68.8 31.3***	4.600**	0.028***

Notes: 1*1=one tablet 50 mg once daily, 1*2=one tablet 50 mg twice daily, 1*3=one tablet 50 mg three times daily. a, b: Variables sharing same letters are not statistically differ from each other. [a]D/C and resume: discontinue AZA and resume again during the treatment course. [b]Treatment shift: shift AZA to another drug. Others= dermatomyositis: two patients, polymyositis: two patients, Sjogren's syndrome: two patients, Wegner's granulomatosis, Cogan's syndrome, and Takayasu arteritis one patient for each disease. **Analysis of variance test, Chi-squared test or Fisher's exact test as appropriate, variables sharing different letters are statistically different from each other. P-value set significant at 0.05 levels. ***Difference is statistically significant.

Abbreviations: LFTs, liver function tests; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis.

another study that concluded that patients who inherit two nonfunctional *TPMT* alleles are at 100% risk for life-threatening bone marrow suppression and that heterozygous patients cannot tolerate full doses of AZA.[30,31] This current study showed that females had a higher risk of immunosuppression than males (*p*-value 0.031). This finding is supported by many other studies that concluded that *TPMT* activity increases in children compared with adults, between adults, it is elevated in males rather than in females.[32–34] During patients follow-up, AZA was discontinued in 50% of patients due to ADRs development, while treatment shift occurred in 45% of patients. Another retrospective study was conducted in the UK and included 113 patients with inflammatory bowel disease mentioned that AZA discontinuation has resulted from AZA adverse effects.[35] In this current study, about 94% of patients initiated on a total of 100 mg dose (<2 mg/kg) and 75% of patients could not tolerate a maintenance dose more

Table 5 Myelosuppression Rates in the Different Types of *TPMT* Mutant Alleles

Variables		Myelosuppression				Test	*P*-value
		No=23		Yes=127			
		N	15.33%	N	84.67%		
*TPMT**3 G460A (rs1800460)	Homozygous Heterozygous Wild type	2 20 1	5.9 18.7 11.1	32 87 8	94.1 81.3 88.9	3.392	0.173
*TPMT**2 G238C (rs1800462)	Homozygous Heterozygous Wild type	0 3 20	0.0 33.3 16.4	19 6 102	100.0 66.7 83.6	6.136	0.032*
*TPMT**3 A719G (rs1142345)	Homozygous Heterozygous Wild type	1 19 3	3.7 17.8 18.8	26 88 13	96.3 82.2 81.3	3.441	0.153
Sex	Male Female	10 13	27.8 11.4	26 101	72.2 88.6	5.651	0.031*

Notes: *P*-value set significant at 0.05 levels. *Difference is statistically significant.

than this dosing range. Our findings were supported by another study which concluded that there was a good clinical response in patients who received low doses of azathioprine (<2 mg/kg). Relapse or lack of response was associated with using of low-AZA dose in patients with high *TPMT* activity.[36]

Conclusion

The study provided an overview of the genomic variations in the Egyptian population. The therapy should be started with a low dose of azathioprine, slowly increase the dose—under close blood counts—until an optimal effect is achieved without the occurrence of side effects.

Routine TPMT genotyping prior to the initiation of AZA therapy may be helpful for Egyptian patients in order to determine whether the drug is appropriate for each patient. Further studies should be conducted to compare the cost-effectiveness of prior screening before starting treatment and treating patients without screening in the Egyptian population. Genotyping will likely provide great pharmacologic and economic benefits in countries like Egypt.

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Disclosure

The authors report no conflicts of interest in this work.

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